4. INTRODUCTION

This protocol is designed to investigate the use of a vaccination approach in the treatment of low-grade human B-cell lymphomas. Previous work has shown that active vaccination of patients with idiotype (Id) protein can induce an anti-idiotype immune response (1). Patients developing an anti-Id immune response had a remission of longer duration and a prolonged survival compared to the patients without immune response and compared to historical control patients (2). Unfortunately, the process of producing the Id proteins needed for a customized vaccine is difficult and time consuming, and adequate vaccine protein production is achieved in only about 80% of the cases. Also, the adjuvants needed for optimal effect of the vaccine often cause substantial side effects. These limitations prevent widespread use of this promising vaccine approach. Therefore, new techniques are needed to induce a tumor-specific immune response in patients.

Research has shown that plasmid DNA injected into skeletal muscle is taken up by cells which then produce the specific protein encoded by the plasmid. Using this technique, investigators in Dr. Ronald Levy's lab at Stanford University have shown that animals vaccinated with plasmid coding for tumor-specific idiotype were protected against subsequent tumor challenge. It was learned that the variable regions of the tumor immunoglobulin had to be linked to xenogeneic constant regions or to cytokine fragments to be effective (3). Preclinical studies have shown that use of DNA encoding an Id/GM-CSF (idiotype/granulocyte macrophage-colony stimulating factor) fusion protein increased immunogenicity in a murine model. Although GM-CSF was not required for DNA immunization, its addition to the construct resulted in earlier antibody responses against the tumor idiotype and yielded a greater proportion of responsive mice than did immunization with idiotype alone. Constructs containing syngeneic constant regions as well as constructs containing the variable regions alone (as scFv) were not protective as DNA vaccines. No adverse effects of these immunizations were observed.

In this Phase I/II protocol, we propose to immunize low-grade non-Hodgkin's B-cell or mantle cell lymphoma patients with a plasmid coding for their specific tumor idiotype linked to murine immunoglobulin constant regions. Patient tumor cells will be harvested at Stanford University Medical Center by the study investigators and sent to Vical Inc. for production of the specific plasmids. The specific objectives of this study are to evaluate (a) the safety and toxicity of the therapy (Vaxid alone and Vaxid plus human GM-CSF (hGM-CSF) plasmid DNA), (b) the induction of an immune response against the idiotype of the tumor immunoglobulin alone and potentially enhanced with hGM-CSF plasmid DNA, and (c) any anti-tumor effect.

5. BACKGROUND AND RATIONALE

5.1 General Information

The two major forms of non-Hodgkin's lymphoma are low and intermediate grade. Most non-Hodgkin's lymphoma cases are in an advanced stage at the time of diagnosis and,

therefore, require a form of systemic therapy. An important advance in the therapy of non-Hodgkin's lymphoma was made in the 1970's with the introduction of Adriamycin-containing combination chemotherapy such as CHOP (cyclophosphamide, hydroxyldaunomycin [Adriamycin], vincristine and prednisone). Complete response rates of 66% for stage III and IV disease with long-term survival rates of approximately 30% were initially reported. However, although low-grade lymphomas are often responsive initially to chemotherapy, they are incurable by current treatment and patients will eventually die of their disease.

Follicular lymphomas of low-grade comprise about 25% of the non-Hodgkin's lymphomas diagnosed annually in the U.S. (4). These lymphomas, including mantle cell lymphoma, are characterized by a relatively slow growth rate and excellent initial response to chemotherapy and radiation therapy (5). Chemotherapy, usually employing alkylator drugs such as chlorambucil or cyclophosphamide, is the standard first line therapy for follicular lymphoma. While complete remission rates as high as 80% have been reported (6), there is a continuous pattern of relapse and no curative therapy has been identified (7). Median survivals range from 4-6 years for mantle cell lymphoma and 6-10 years for other low-grade non-Hodgkin's lymphomas. Over the course of the disease, histologic progression to a diffuse aggressive lymphoma is commonly observed as well as occasional spontaneous regressions. Overall, there has been no improvement in survival in low-grade lymphoma seen with conventional chemotherapy in the past 30 years. Thus, the ineffectiveness of conventional chemotherapy, coupled with the harmful effects of such treatments, has motivated the search for novel treatment approaches.

Among the newer treatments for follicular lymphoma is interferon alpha, given concomitantly with chemotherapy and/or as an adjuvant (8-10). While this strategy has resulted in a significant delay in the time to progression in several studies, this progress is offset by the considerable side effects of maintenance interferon, and the continuous pattern of relapse. Recently, the activity of the purine analogs, fludarabine and 2-chlorodeoxyadenosine, has been demonstrated in the low-grade lymphomas, particularly follicular small cleaved cell lymphoma (11,12). These agents disrupt intracellular nucleotide pools and have activity in both dividing and resting cells. Increased expression of *bcl-2,* which is typical of the follicular lymphomas, may be associated with resistance to chemotherapeutic agents, including the purine analogs and radiotherapy (13). This observation has further increased interest in biological therapies for the follicular lymphomas.

B-cell malignancies (i.e. lymphoma and leukemia of the B-cell type, and myeloma) are unique in that they are one of the few cancers in which a tumor-specific antigen has been identified. B-cells express an immunoglobulin (Ig) comprised of a heavy chain and a light chain on their surface. An enormous variety of Ig's exists, each with unique variable regions. The specific structure of the variable region expressed by the Ig on the surface of the B-cell is called the idiotype (Id). The idiotype of an Ig refers to the antigenic motifs formed by the combination of the immunoglobulin heavy and light chains. Since the B-cell malignancies are clonal diseases and the malignant clone arises only after the variable region has been defined by Ig gene rearrangement, all the cells of a given B-cell tumor express identical

immunoglobulins and thus can be distinguished from normal B-cells by virtue of their unique Id. Thus, the idiotype of the tumor cell immunoglobulin represents a unique, tumor-specific marker which can be recognized by the immune system and can be used as a target for immunotherapy.

Two therapeutic approaches that have taken advantage of targeting therapy to the idiotype expressed on B-cell lymphomas. The first of these approaches involves administration of anti-idiotypic monoclonal antibodies directed against the unique idiotype expressed by the patient's lymphoma (passive immunotherapy). The treatment with monoclonal anti-Id antibodies has been reported to result in long lasting remissions (14-16). It is known from previous therapies utilizing murine monoclonal antibodies that the possible induction of human anti-mouse antibodies (HAMA) produces no adverse effects in patients (17,18). The second approach consists of an active immunotherapy involving vaccination of the patient with the idiotype expressed by the patient's lymphoma. This results in the induction of an immune response against the idiotype expressed by the patient's lymphoma. In clinical trials, Levy and colleagues have demonstrated clinical efficacy in B-cell lymphoma patients following customized idiotypic vaccination (1, 2). Despite the recent encouraging results with both anti-idiotypic monoclonal antibody therapy and idiotypic protein vaccination, the clinical applications of these types of therapies are limited by the time and effort required to obtain the patient's idiotypic Ig. Both of these therapies require the preparation of hybridomas from patient biopsies, in vitro culture, identification, and selection of clones expressing Ig of the same immunophenotype as the tumor, expansion of these clones in culture, and finally purification of sufficient quantities of the idiotypic Ig to be used to vaccinate the patient in the case of active immunotherapy or to be used to generate anti-idiotypic monoclonal antibodies in the case of passive anti-idiotypic monoclonal antibody therapy. Furthermore, there are often substantial side effects associated with the adjuvants needed for optimal effect of the vaccine. These factors currently prevent a more widespread application of this promising vaccine approach. New strategies for idiotype vaccination are therefore highly desirable.

Genetic vaccination with plasmid DNA expression vectors containing the coding sequence for a patient's specific B-cell lymphoma idiotype offers a simpler, more efficient alternative route. DNA vaccines provide for production of the relevant immunogen *in vivo* without using live agents and have been shown to induce a protective immune response for viral (19-27), bacterial (28,29) and parasitic (30) diseases. Only the protein encoded by the injected DNA is expressed in cells. Thus, DNA immunizations mimic live-attenuated vaccines by producing the encoded protein within the hosts' cells. This allows processing and folding of the gene product into a native structure leading to an effective target antigen. The positive aspects of immune stimulation inherent in live-attenuated vaccines are combined with the safety of recombinant subunit vaccines in an adjuvant-free formulation. Plasmid DNA constructs are technically easier and less time consuming to prepare and test than recombinant proteins. This is especially important in the case of a customized vaccine preparation. Conventional fermentation facilities are utilized in the same way that recombinant proteins are produced, permitting easy manufacturing scale-up.

Recent technological developments have made a genetic approach to idiotypic therapy possible. The utility of polymerase chain reaction (PCR) technology has allowed for the rapid cloning of the variable domains from Ig genes (31-33). This technology has been used to identify and clone the variable domain genes from the idiotypic Ig of malignant B-cells from lymphoma biopsies (34,35). Vaccination of mice with plasmid DNA expression vectors containing the coding region for murine B-cell lymphoma idiotypes has been shown to elicit anti-idiotypic antibodies (36) and protect mice from challenge with the idiotype expressing B-cell lymphoma (3,37).

GM-CSF is best known for its ability to stimulate the proliferation and differentiation of leukocyte hematopoetic precursor cells. However, it also has powerful potentiation effects on the immune response. GM-CSF induces localized inflammation at the site of injection, associated with its ability to chemo-attract and activate neutrophils/macrophages and elicit the secretion of inflammatory cytokines, including TNF-α, IL-1, IL-6 and IL-12. As part of leukocyte activation, antibody-dependent cellular cytotoxicity is enhanced. GM-CSF also increases MHC class II, and B7-1 (CD80) and B7-2 (CD86) T-cell co-stimulator-y molecule expression on the surface of antigen-presenting cells such as macrophages and dendritic cells.

In this Phase I/II protocol, we will produce a specific customized idiotype-expressing plasmid DNA and use it to immunize patients, with and without coinjected plasmid DNA encoding hGM-CSF, with the expectation that their immune systems will produce a specific anti-Id immune response and protect against relapse, therefore prolonging survival.